An update on the minimal cell project: From the physics of solute encapsulation to the experimental modeling of cell communities.

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Abstract

The minimal cell (MC) project aims at understanding the emergence of cellular life by constructing experimental models of cells, according to a synthetic (constructive) biology approach. Our strategy – also known as the semi-synthetic one – is based on the encapsulation of the minimal number of biomolecular components inside lipid vesicles (liposomes). Being interested in studying the key step for constructing semi-synthetic cells, namely the physical entrapment of the solutes, we have recently reported that the mechanism of vesicle formation can lead to a spontaneous local increase in concentration of proteins inside vesicles (Luisi et al., ChemBioChem 2010, 11, 1989-1992). In particular, it was shown that the protein ferritin can reach intravesicle concentration of at least one order of magnitude higher when compared to the bulk (external) concentration. This self-organization phenomenon might give a rational account for the formation of functional cells from diluted solutions, and therefore help to understand the origin of metabolism. The effective encapsulation of solutes, however, is only one of the ways for achieving functional cells. The second route is fusion of vesicles or the exchange of solutes among vesicles (Caschera et al., J. Coll. Inter. Sci. 2010, 345, 561-565). Both processes allow the combination of different solutes to give compartments that can exhibit improved reactivity. Aiming at developing a realistic model for cooperative interactions among vesicles, we have recently developed a cell colony model. This is based on the formation of lipid vesicles clusters adherent to a solid substrate, representing a minimal model of cell communities. Here we summarize the most significant aspects of our recent activities.

The physics of solute encapsulation

Looking at the physico-chemical mechanisms that have lead to the origin of cellular life, a still open question is whether functional cells have been originated from the encapsulation of an already developed metabolism (metabolism- or replicator-first scenarios), or whether the cell metabolism was entirely (or almost entirely) developed inside compartments (compartment-first scenario). In both cases, there are some aspects that need clarification, as the low probability of co-entrapping all required molecules in the same compartment in the first hypothesis, or the lack of permeability control in the second hypothesis (Luisi et al., 2010).

In particular, although the encapsulation of solutes into liposomes is a well-established field, especially due to the large amount of work done in the field of drug delivery, we still miss a complete view of the physics underlying this important mechanism. In fact, with a few exceptions (Sun and Chiu, 2005; Dominak and Keating, 2007; Lohse et al., 2008), all experimental studies deal with the *average* entrapment yield, and no attention has been given to the entrapment behavior at the level of single vesicles, also due to technical difficulties.

We have recently started a systematic study on the encapsulation of biopolymers into lipid vesicles. This study was inspired by our report on the protein expression inside 200 nm (diameter) vesicles, that suggested the possible deviation from the expected intravesicle solute distribution (Souza et al., 2009).

As a model system, we have used the protein ferritin, an iron-storage protein, consisting of a nucleus of electron dense ferrihydrite-like iron salts surrounded by 24 protein subunits. Ferritin can be directly visualized as single molecule by electronmicroscopy, so that it becomes possible to directly count the number of ferritin molecules inside vesicles imaged via cryo-transmission electron microscopy.

After analyzing about 7,700 submicrometric vesicles (Fig. 1a), prepared by varying the concentration of ferritin, the preparation method and the membrane lipid composition, we have concluded that the encapsulation of this solute inside lipid vesicles does not follow the expected behavior. In our experimental conditions, this is given by the Poisson distribution of N solutes inside vesicles that are expected to entrap, on average, μ solutes:

$$f(N) = e^{-\mu} \frac{\mu^{-N}}{N!}$$

where $f(N)$ represents the fraction of vesicles containing N ferritins, and μ is the average expected number of ferritin molecules. The μ value can be calculated from the vesicle volume V, and the ferritin concentration C:

$$\mu = N_A C \cdot V$$

(N_A being the Avogadro's number).

In particular, we have found that the distribution of ferritin-containing vesicles follows a power-law-like shape, characterized by an abnormally high amount of empty vesicles ($N = 0$), a decreasing pattern at intermediate N, and – significantly – a non-zero long tail (Fig. 1b), which represents

the non negligible probability of co-entrapping a relatively high number of ferritins (up to hundreds), whereas the average expected value is of few units.

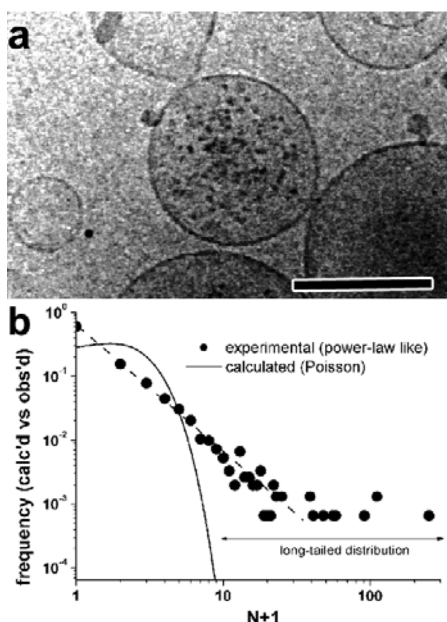

Figure 1. Entrapment of ferritin inside lipid vesicles. (*a*) Cryo-TEM electronmicrograph of a ferritin containing vesicle (size bar 200 nm). (*b*) Comparison between calculated Poisson (C = 4 μM, *diam.* 100 nm) distribution and experimental data profile. Redrawn after Luisi et al. (2010). The "long tail" feature has been highlighted. Note the logarithmic axes and the abscissa values shift (N+1).

According to these results it appears that the co-encapsulation of several molecules in the same compartments is a physically possible process, and we believe that these observations contribute significantly for understanding the emergence of complex primitive cells from separated components. In fact, our results demonstrate that it is possible to form a solute-rich compartment even starting from diluted solution. This also implies that sluggishly reacting (diluted) systems might become reactive thanks to the spontaneous concentration increase inside lipid vesicles. Further studies about the mechanism will clarify our work hypothesis, based on weak and cooperative solute/membrane interactions, which affect the mechanism of vesicle closure (i.e., a process under kinetic control).

Experimental models of cell communities

As we have anticipated, the co-entrapment of diverse solutes in the same compartment is not the unique process that can lead to solute-rich compartments starting from simpler ones. A complementary way is represented by all those mechanisms that have as a result the sharing of solutes among several compartments, in particular fusion and solute exchange. We have recently reported a study on the fusion between cationic and anionic vesicles as a way for reaching higher complexity, and loosely resembling the idea of symbiogenesis. In particular, it was shown that oppositely charged vesicles can react (up to ~ 20% yield) to neutralize their net charge and give rise to neutral species derived from the fusion of the vesicles (Caschera et al., 2010). As a consequence, the internal solutes, initially present in two vesicle populations, become co-encapsulated in the resulting new vesicles. We have reasoned that such fusion process, as well as the possibility of exchanging solutes among vesicles, could occur not only in suspended vesicles, but also in the case of vesicles forming small solid-supported communities. Here, the physical proximity of vesicles could not only favor such dynamical transformation, but simultaneously stabilize the community thanks to multiple physical interactions. Research is currently going on in our laboratory aimed at characterizing vesicle colonies with respect to their reproducible formation, physico-chemical stability, fusion, solute exchange as well as solute capture from the environment, and stability against flow (Carrara, 2010).

Thanks to this new experimental model we aim at studying the new "dimension" of *cell communities*, which is generally missing in the discussion on the origin of cellular life. Moreover, the model will allow a more direct investigation of communication between synthetic cells through the synthesis, release, uptake, and processing of diffusible species. This represents a concrete example of chemical communication, with possible implication in chemically-based information and communication technologies (ICTs).

A first attempt to use lipid vesicles for establishing a communication between synthetic and natural cells has been reported by Gardner et al. (2009).

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